Serial No. 10/082,034 Dkt. No. 6680.040
Title: SOLUBLE HLA LIGAND DATABASE UTILIZING PREDICTIVE ALGORITHMS AND METHODS OF MAKING AND USING SAME
Applicant: William Hildebrand Group No.: 2171
Filed: 02/21/2002 Examiner: Unknown
Atty: Douglas J. Sorocco Tel: (405) 478-5344
Page 1 of 17

Reverse phase HPLC of class I HLA eluted peptide ligands

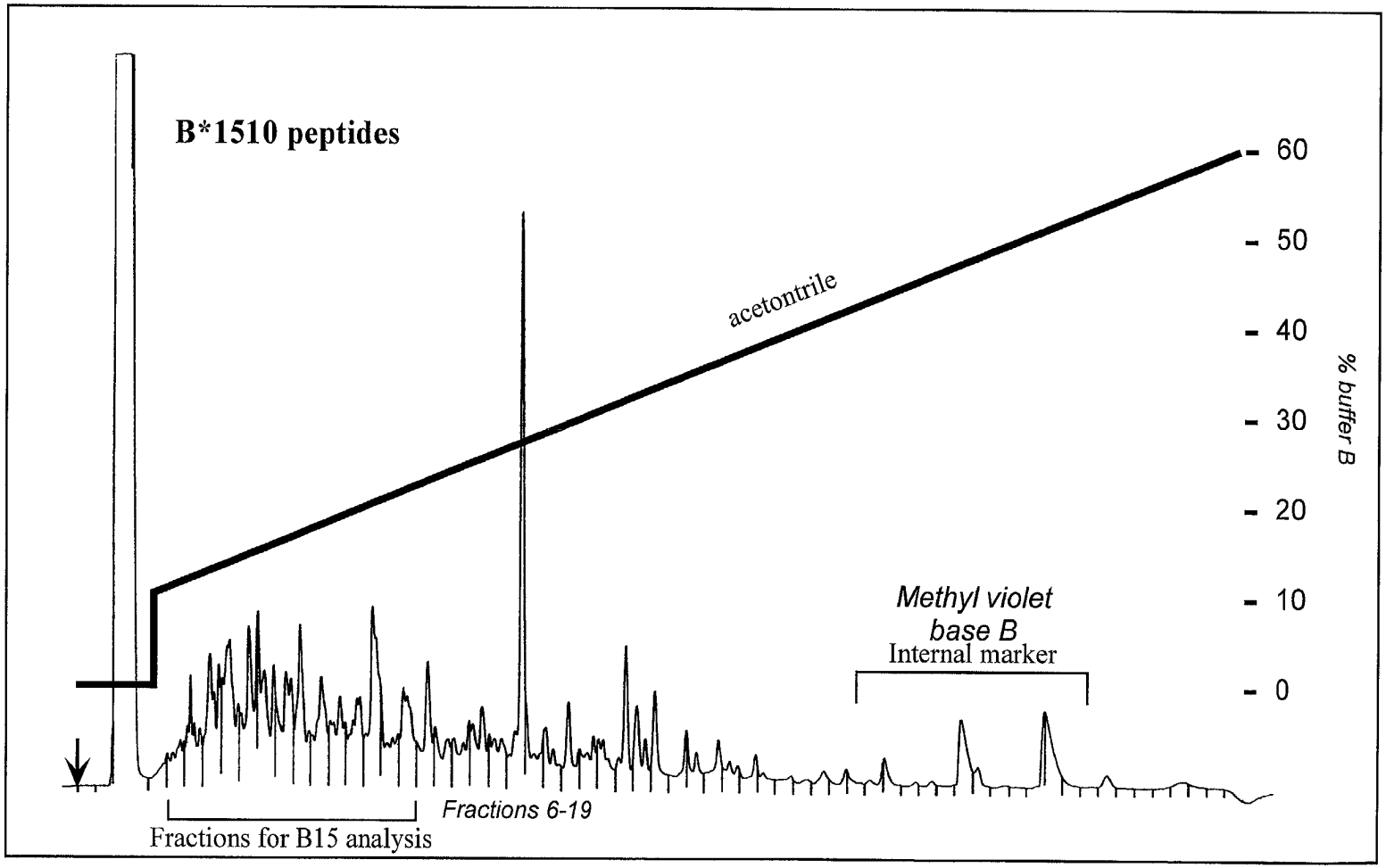


Fig. 1

Ion maps of peptides eluted from various B15 class I sHLA molecules. Mapping was accomplished with a nano-spray needle and an ESI mass spectrometer. The figure shows that the same ion peak is present in 3 of 4 B15 class I.

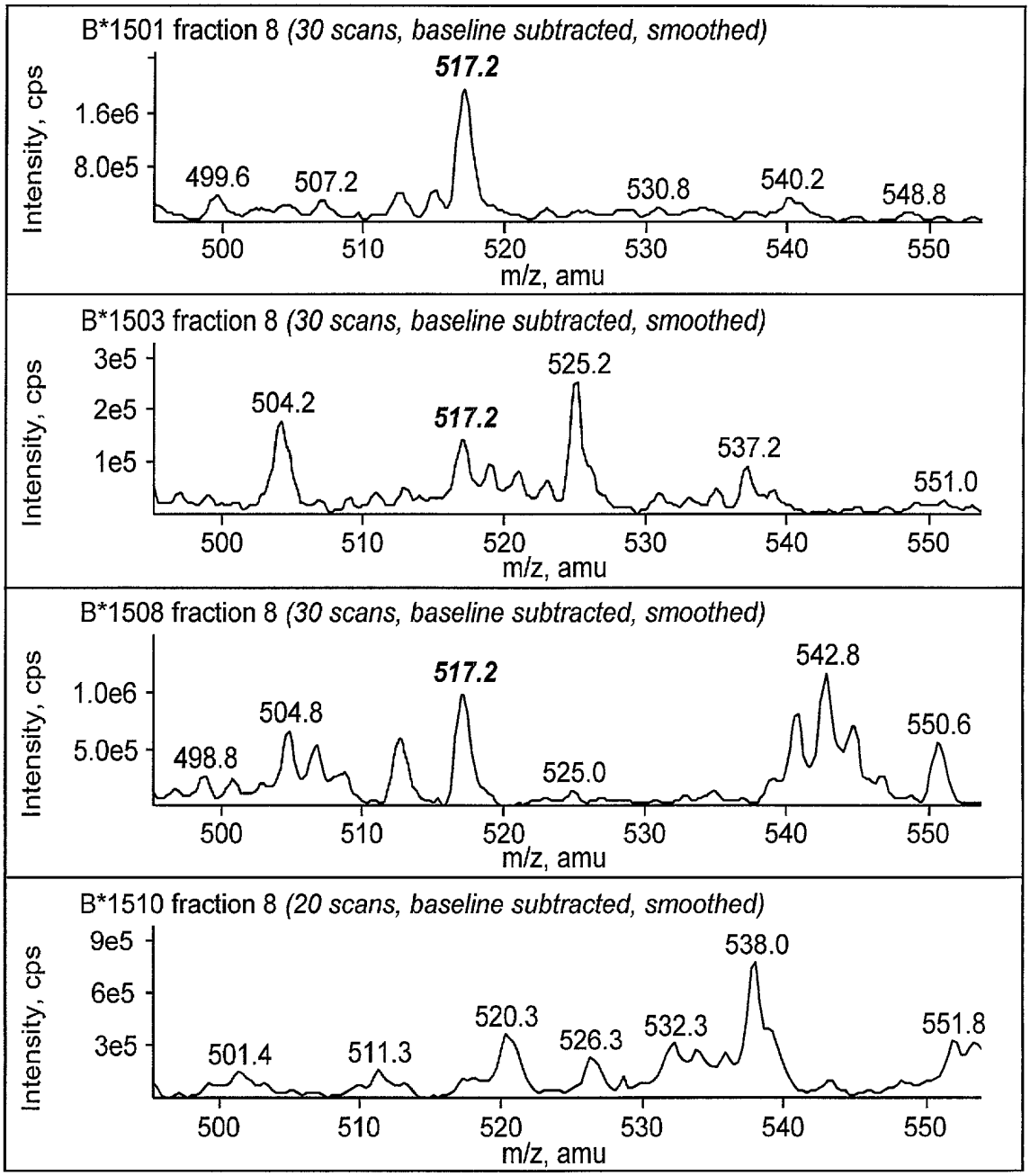


Fig. 2

Serial No. 10/082,034 Dkt. No. 6680.040
Title: SOLUBLE HLA LIGAND DATABASE UTILIZING PREDICTIVE ALGORITHMS AND METHODS OF MAKING AND USING SAME
Applicant: William Hildebrand Group No.: 2171
Filed: 02/21/2002 Examiner: Unknown
Atty: Douglas J. Sorocco Tel: (405) 478-5344

MS/MS fragmentation-sequencing of ion 517.2 from the various B15 class I sHLA molecules. This data was accomplished by completing a second nanospray of the peptides in fraction 8 from the HPLC. This demonstrates how ions can be MS ion mapped and subsequently MS/MS sequenced. There is sufficient peptide present to do multiple MS/MS fragmentation runs. There is also sufficient peptide present to facilitate a submotif on fraction 8 or further separation in the event that two peptides had mapped at 517.2 in the ion map.

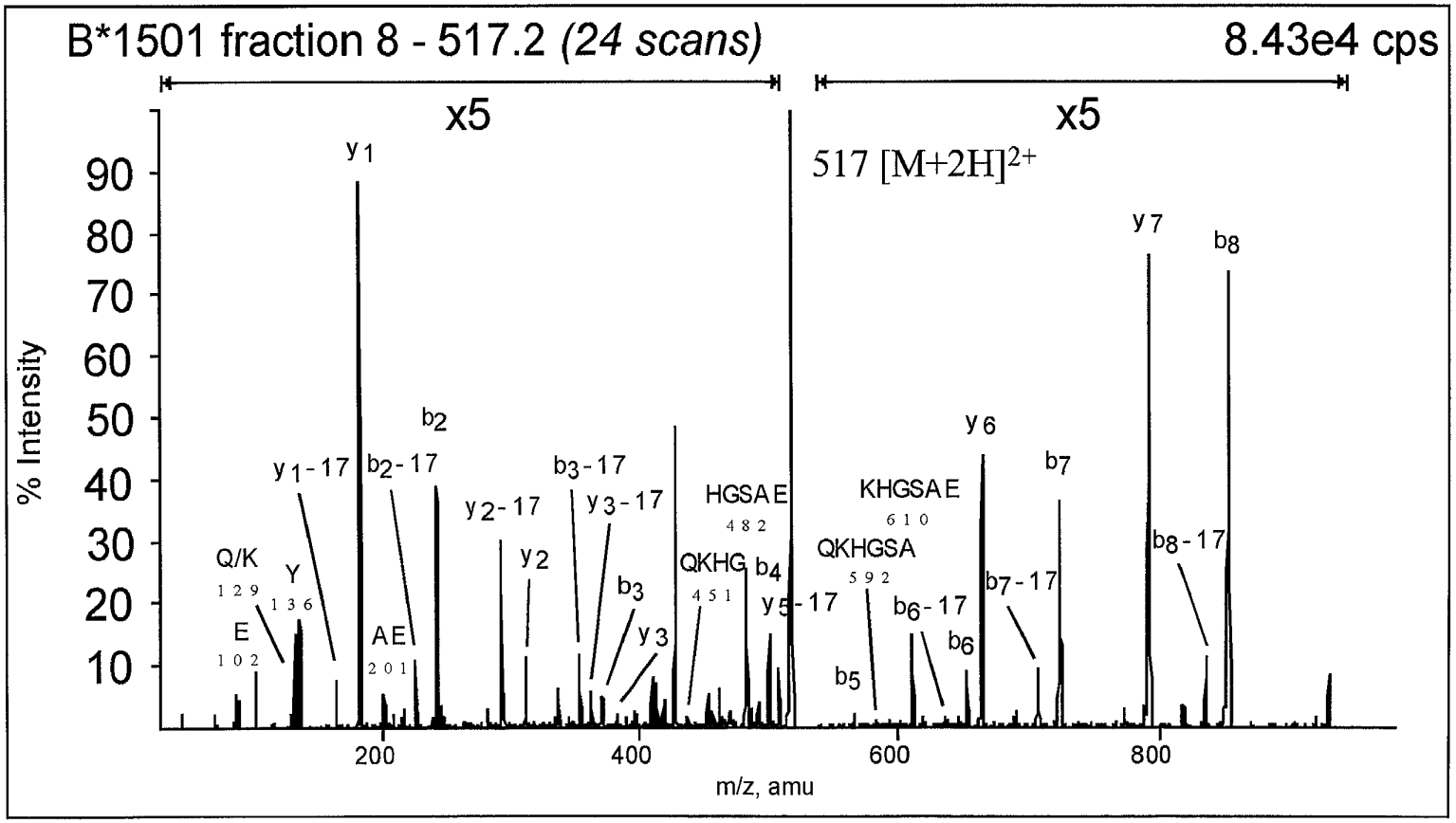


Fig. 3 1 of 3

20 52 50 "42 20 28 04 1

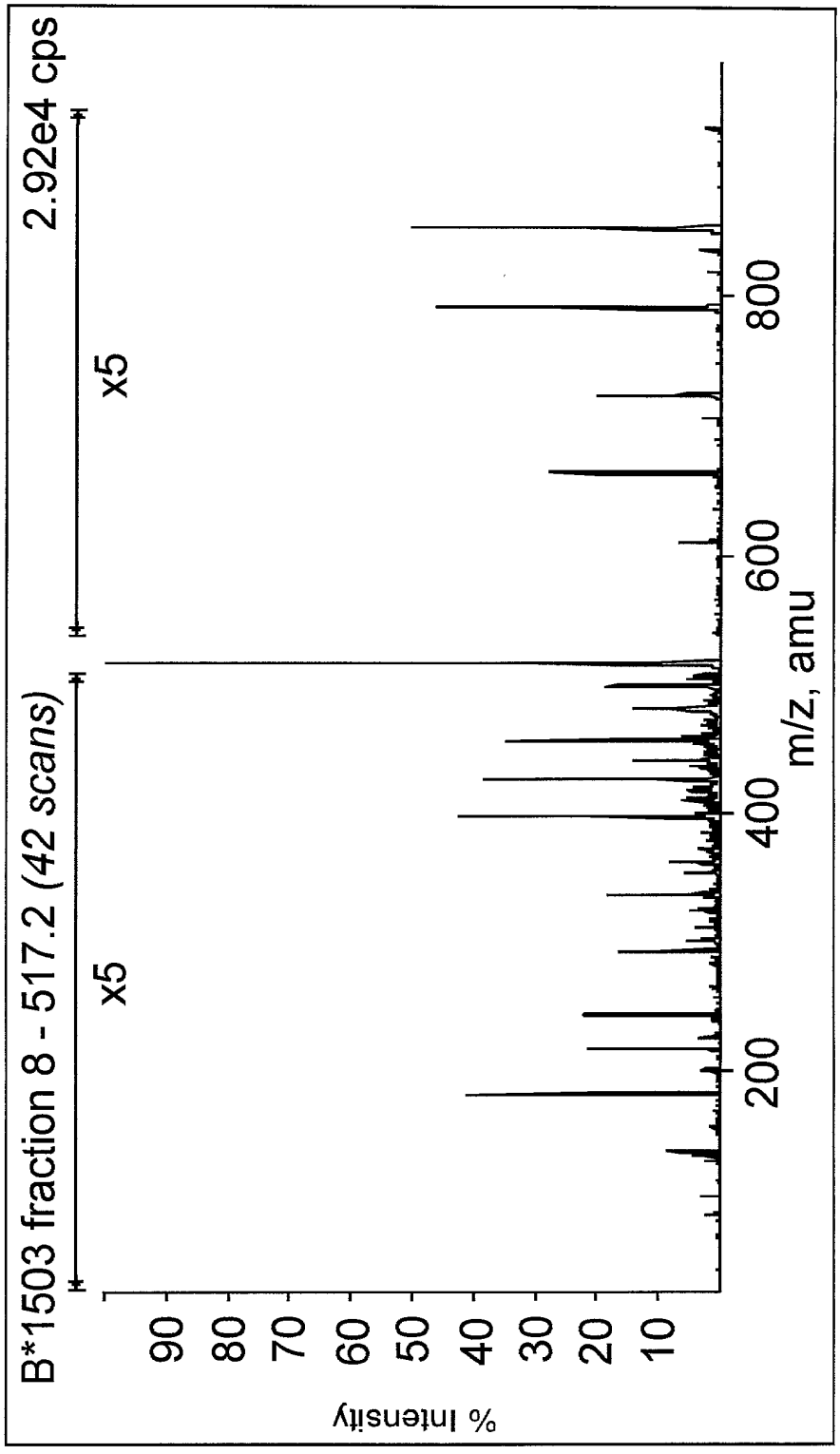


Fig. 3 2 of 3

20525b-1p.ct.2.8.04

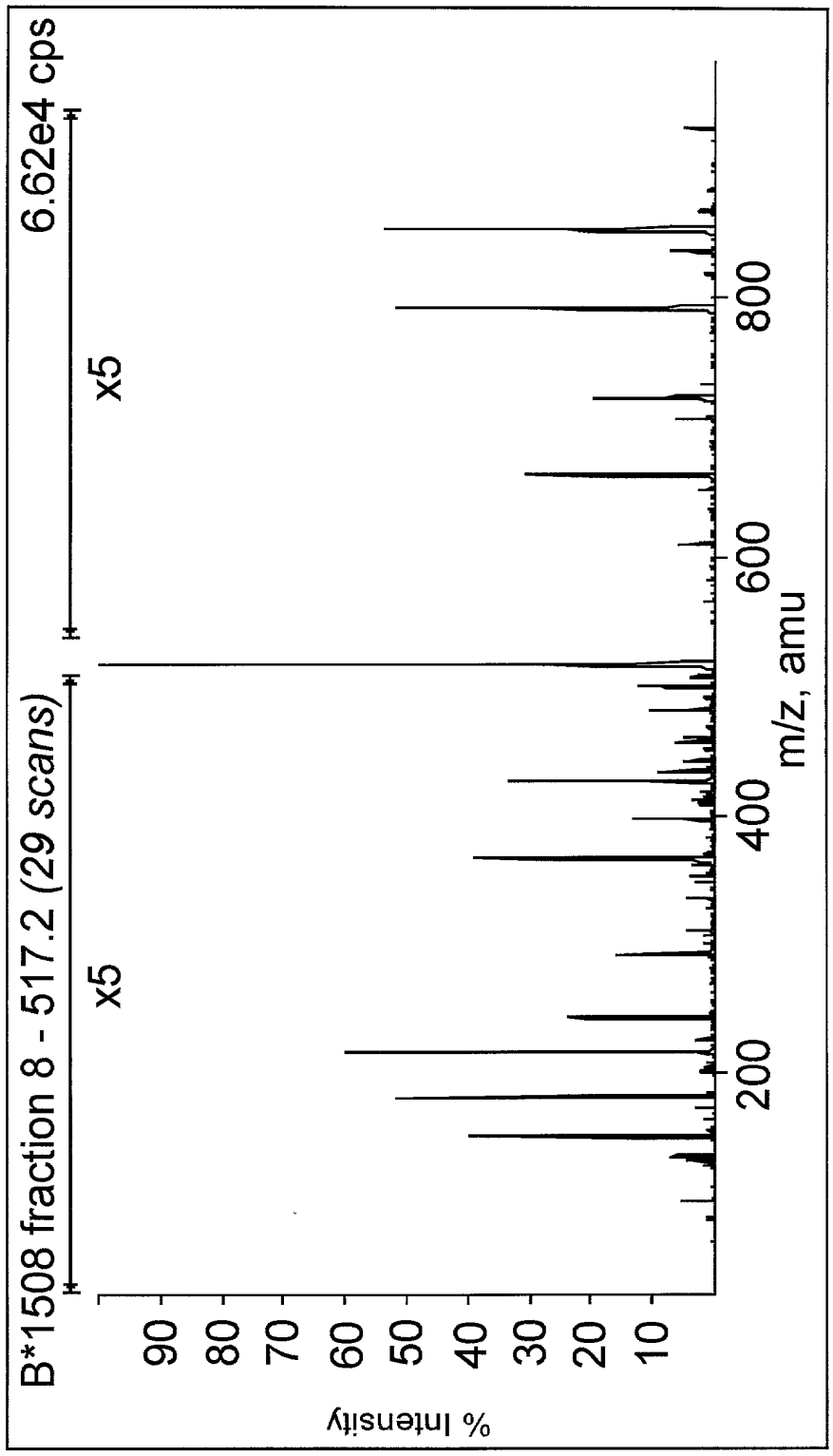
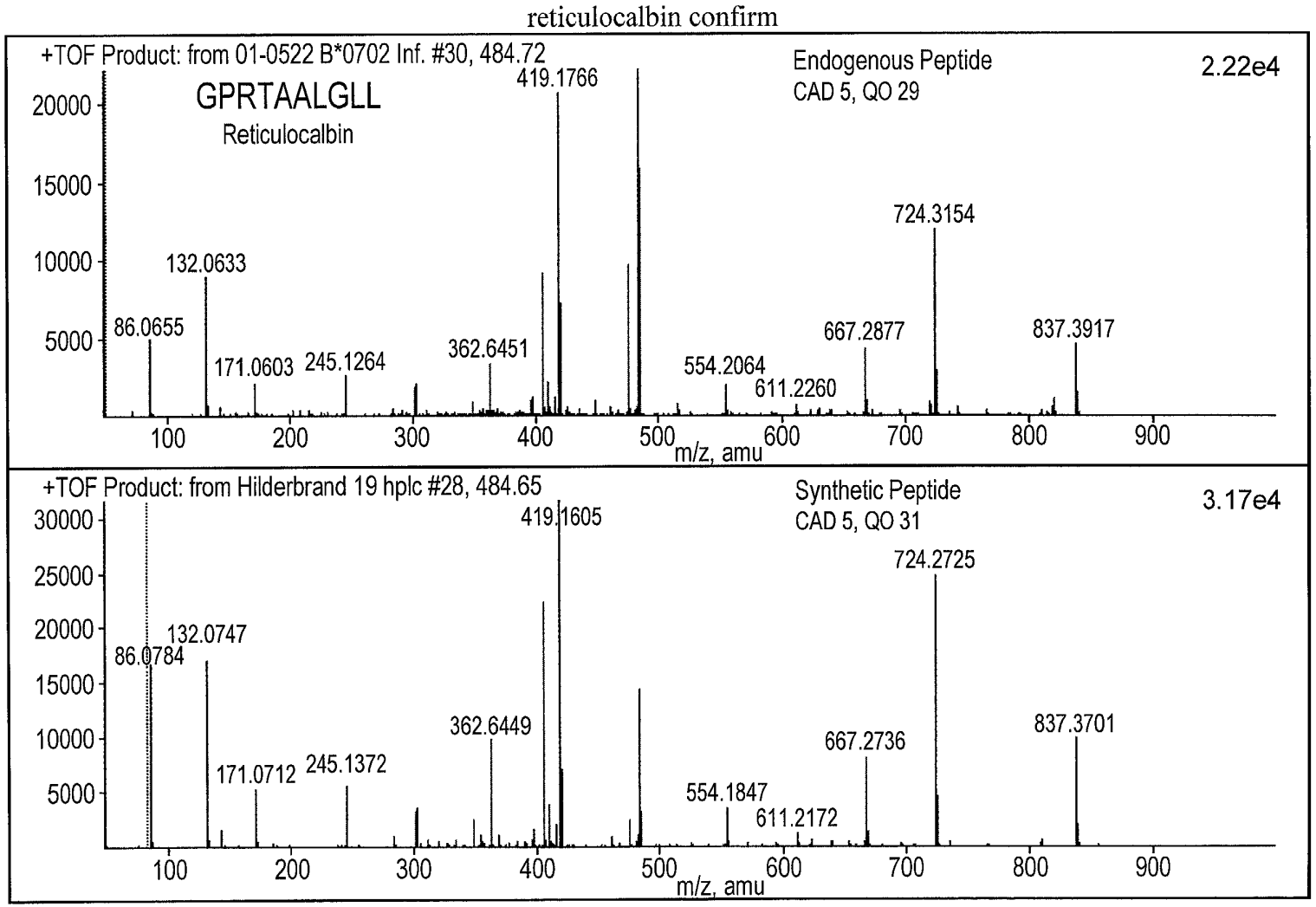


Fig. 3 3 of 3

209290 "hE028007F

Serial No. 10/082,034 Dkt. No. 6680.040
Title: SOLUBLE HLA LIGAND DATABASE UTILIZING PREDICTIVE ALGORITHMS AND METHODS OF MAKING AND USING SAME
Applicant: William Hildebrand Group No.: 2171
Filed: 02/21/2002 Examiner: Unknown
Atty: Douglas J. Sorocco Tel: (405) 478-5344
Page 6 of 17



sHLA B*0702 was secreted from HIV infected and uninfected cells. The ion maps of the peptides eluted from sHLA B*0702 in infected and uninfected cells were compared. Ion 484.72 was unique to the HIV infected cells. Ion 484.72 was subjected to MS/MS fragmentation-sequencing. We called GPRTAALGLL as the sequence of the ligand. We synthesized this peptide and found that it generated the same MS/MS fragmentation pattern as the ligand from HIV infected cells. This MS/MS data on a synthetic ligand matches our experimental data and validates the accuracy of our sequence.

Fig. 4

B*1508

	1	2	3	4	5	6	7	8	9
dominant	-	P	N	-	-	-	-	-	Y
		A	F						
			K						
			R						
			H						
			Y						
			I						
strong	-	-	-	E	H	R	-	-	F
				D					
				G					
				S					

B*1501

	1	2	3	4	5	6	7	8	9
dominant	-	Q	K	-	-	-	-	-	Y
			F						
			N						
			R						
			Y						
strong	-	M	P	P	G	-	-	-	F
		L	H	D	I				
		V	A	G					
				E					

B*1510

	1	2	3	4	5	6	7	8	9
dominant	-	H	Y	P	-	-	-	-	L
			A						
			I						
			K						
			S						
			F						
			L						
			V						
			T						
strong	-	-	G	R	R	I	M	-	F
			D	D	V		L		
			E	K					
			M	E					
			N						

Fig. 5

Fig. 5 continued

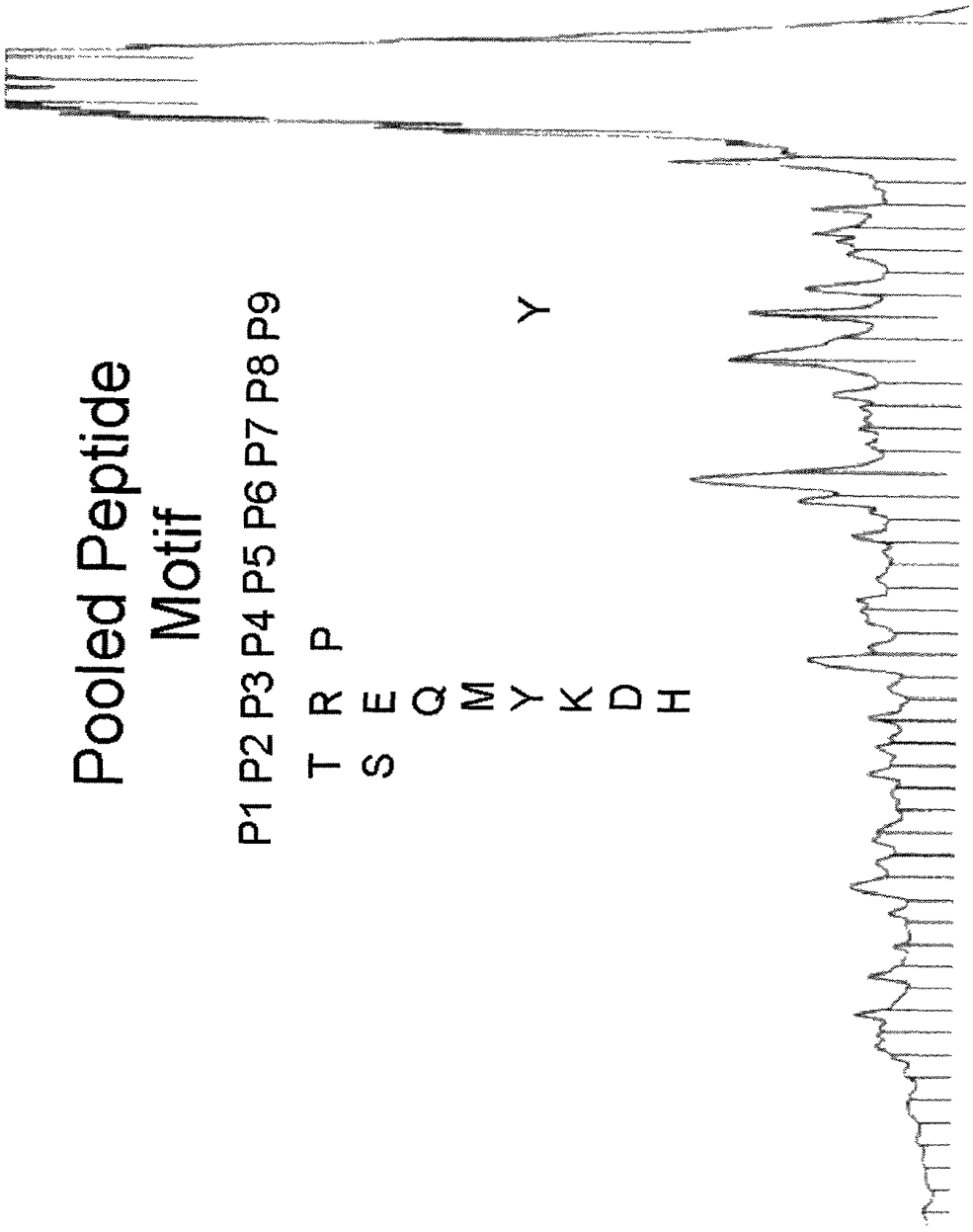


Fig. 6

20020210 "Express Mail" Label No. EL 992 603 034 US

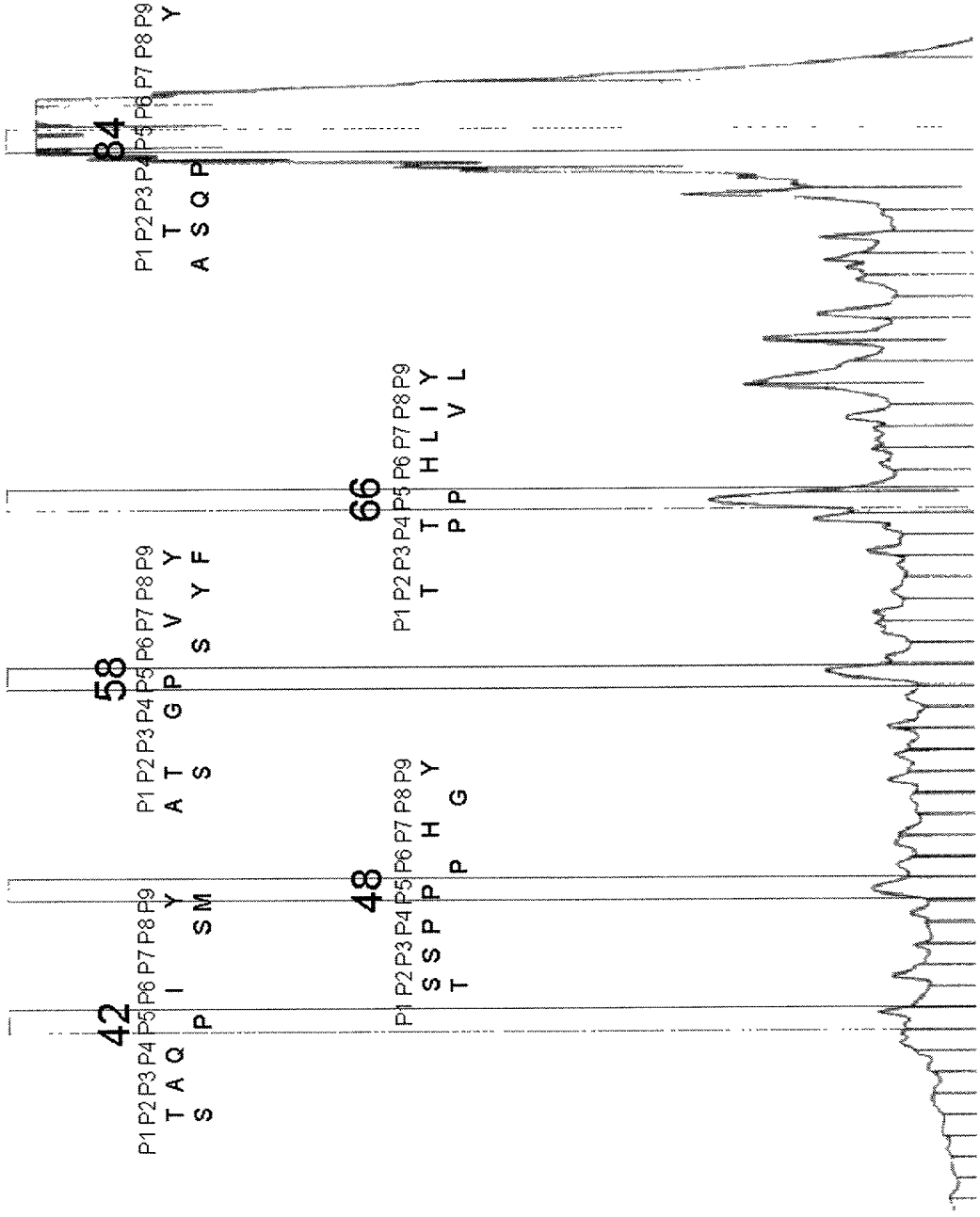

84
P1 P2 P3 P4 P5 P6 P7 P8 P9
T Y
A S Q P
66
P1 P2 P3 P4 P5 P6 P7 P8 P9
T T H L I Y
P P V L
58
P1 P2 P3 P4 P5 P6 P7 P8 P9
A T G P V Y
S S Y F
48
P1 P2 P3 P4 P5 P6 P7 P8 P9
S S P P H Y
T P G
42
P1 P2 P3 P4 P5 P6 P7 P8 P9
T A Q I Y
S P S M

Fig. 7

Narrowing search parameters using fraction motifs:

Ovarian Carcinoma Immunoreactive Antigen

MNGRADFREP	NAEVPRPIPH	IGPDYI**PTEE**	**ERRY**FAECND	**ESFWFRSY**PL
AATSMLITQG	LISKGILSSH	PKYGSIPKLI	LACMGYFAG	KLSYVKTCQE
KFKKLENSPL	GEALRSGQAR	**RSSPPGHYY**Q	KSKYDSSVSG	QSSFVTSPAA
QSSFVTSPAA	DNIEMLPHYE	PIPFSSSMNE	SA**PTGITDHI**	**Y**QGPDPNLEE
SPKRKNITYE	ELRNKNRESY	EVSLTQKTDP	SVRPMHERVP	KKEVKVNKYG
DTWDE				

Scanning with whole-pooled motif revealed 4 putative epitopes.

Ovarian Carcinoma Immunoreactive Antigen

MNGRADFREP	NAEVPRPIPH	IGPDYIPTEE	ERRYFAECND	ESFWFRSYPL
AATSMLITQG	LISKGILSSH	PKYGSIPKLI	LACMGYFAG	KLSYVKTCQE
KFKKLENSPL	GEALRSGQAR	**RSSPPGHYY**Q	KSKYDSSVSG	QSSFVTSPAA
QSSFVTSPAA	DNIEMLPHYE	PIPFSSSMNE	SAPTGITDHI	YQGPDPNLEE
SPKRKNITYE	ELRNKNRESY	EVSLTQKTDP	SVRPMHERVP	KKEVKVNKYG
DTWDE				

Scanning with fraction 48 peptide motif revealed 1 putative epitope.

Fig. 8

20030044807

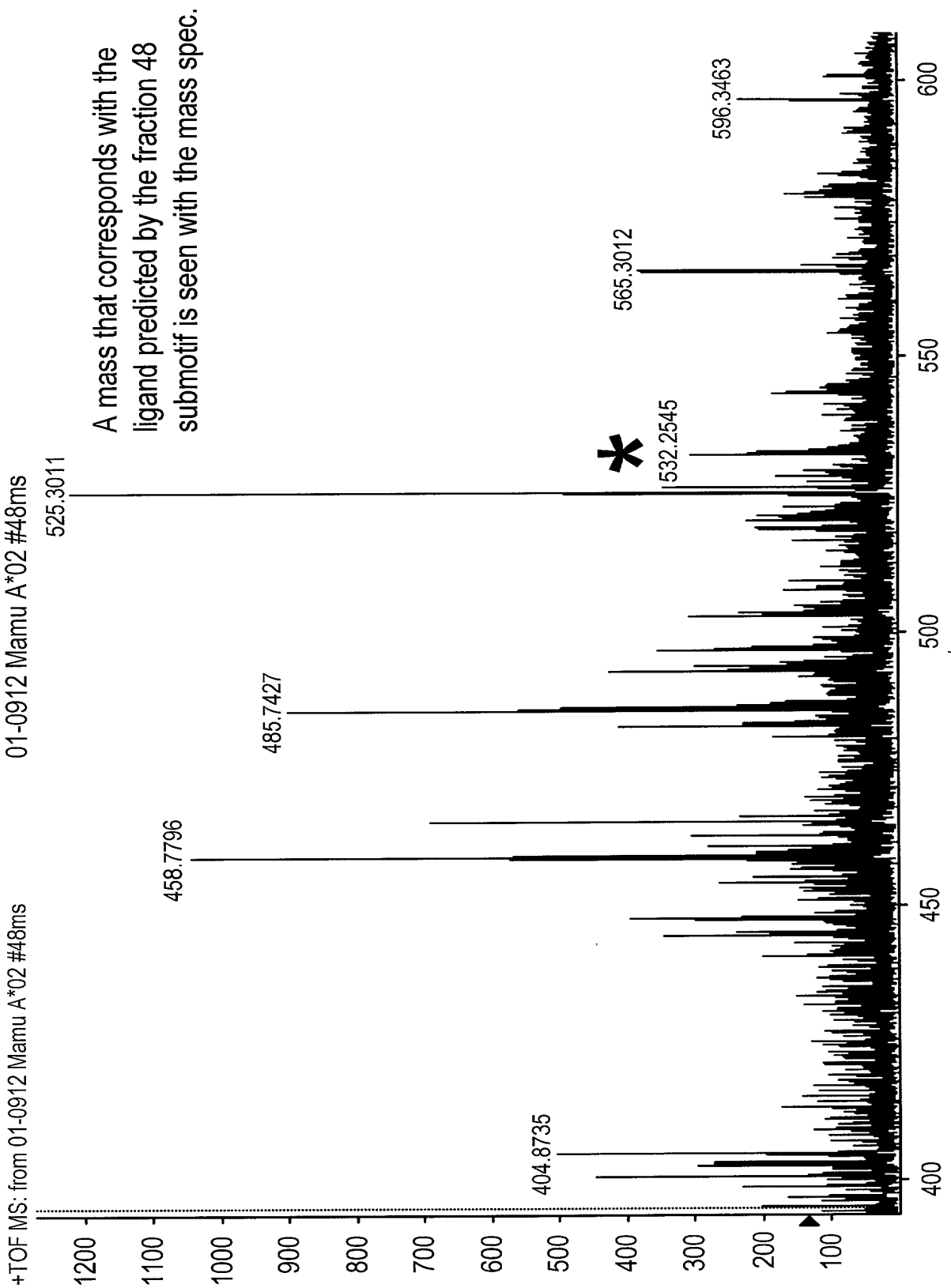


Fig. 9

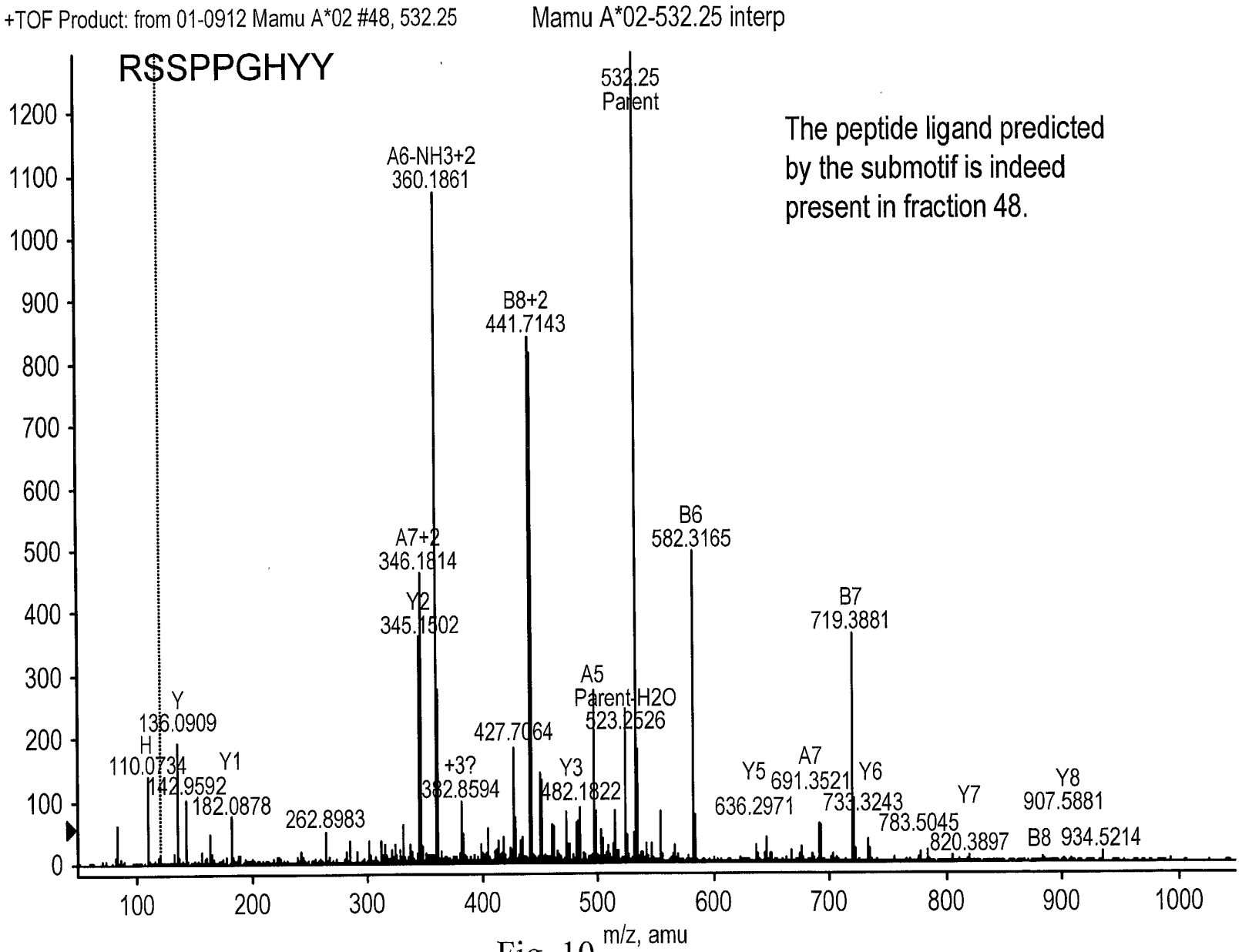

+TOF Product: from 01-0912 Mamu A*02 #48, 532.25
Mamu A*02-532.25 interp
R$SPPGHYY
532.25
Parent
A6-NH3+2
360.1861
B8+2
441.7143
B6
582.3165
A7+2
346.1814
Y2
345.1502
B7
719.3881
A5
Parent-H2O
523.2526
Y
136.0909
H
110.0734
Y1
142.9592
182.0878
262.8983
427.7064
+3?
382.8594
Y3
482.1822
Y5
636.2971
A7
691.3521
Y6
733.3243
Y7
783.5045
820.3897
Y8
907.5881
B8 934.5214
The peptide ligand predicted by the submotif is indeed present in fraction 48.
m/z, amu

Fig. 10

Motif Data (Edman sequencing)

	1	%	fold	2	%	fold	3	%	fold	4	%	fold	5	%	fold	6	%	fold	7	%	fold	8	%	fold	9	%
Dominant 3.5 fold increases or more over prior round							F	9.20	11.18																	
							I	7.60	7.01																	
							N	6.20	4.11																	
							M	4.90	10.42																	
Strong 2.5-3.5 fold increase over prior round	K	31.50		R	53.80	2.57	Q	8.20	3.10	P	8.30	2.97													L	7.50
	R	15.50					K	5.40	2.67																	
	S	10.40					L	5.10	3.47																	
Weak 2.0-2.5 fold increase over prior round							A	5.80	2.08				M	4.90	2.28				T	7.70	2.12					
Trace 1.50-2.0 fold increase over prior round				Q	3.60	1.79	P	2.20	1.94	K	11.00	1.63	I	6.50	1.99							Q	11.40	1.93		
										S	6.00	1.68	F	4.20	1.83							N	5.70	1.55		
										V	5.30	1.99	H	1.80	1.67											

Fig. 11

Design of HLA Ligand/Motif Database

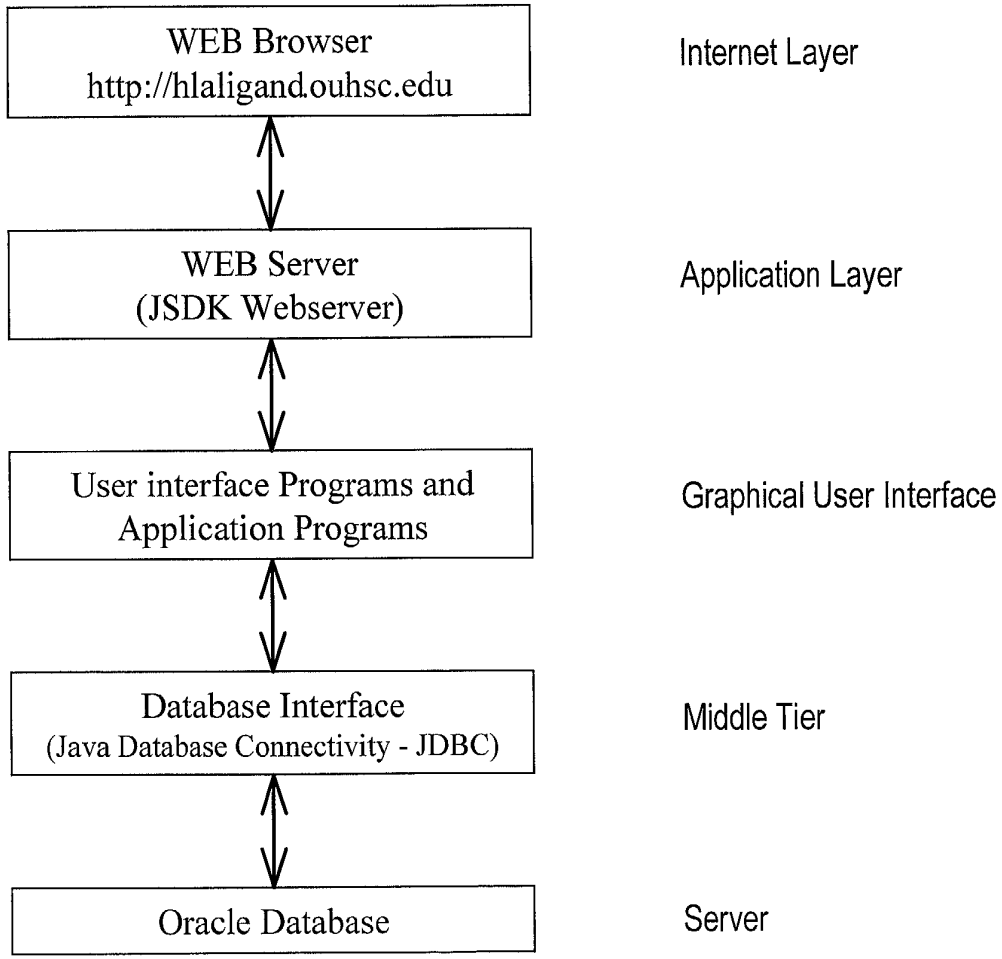


Fig. 12

EXPRESS MAIL

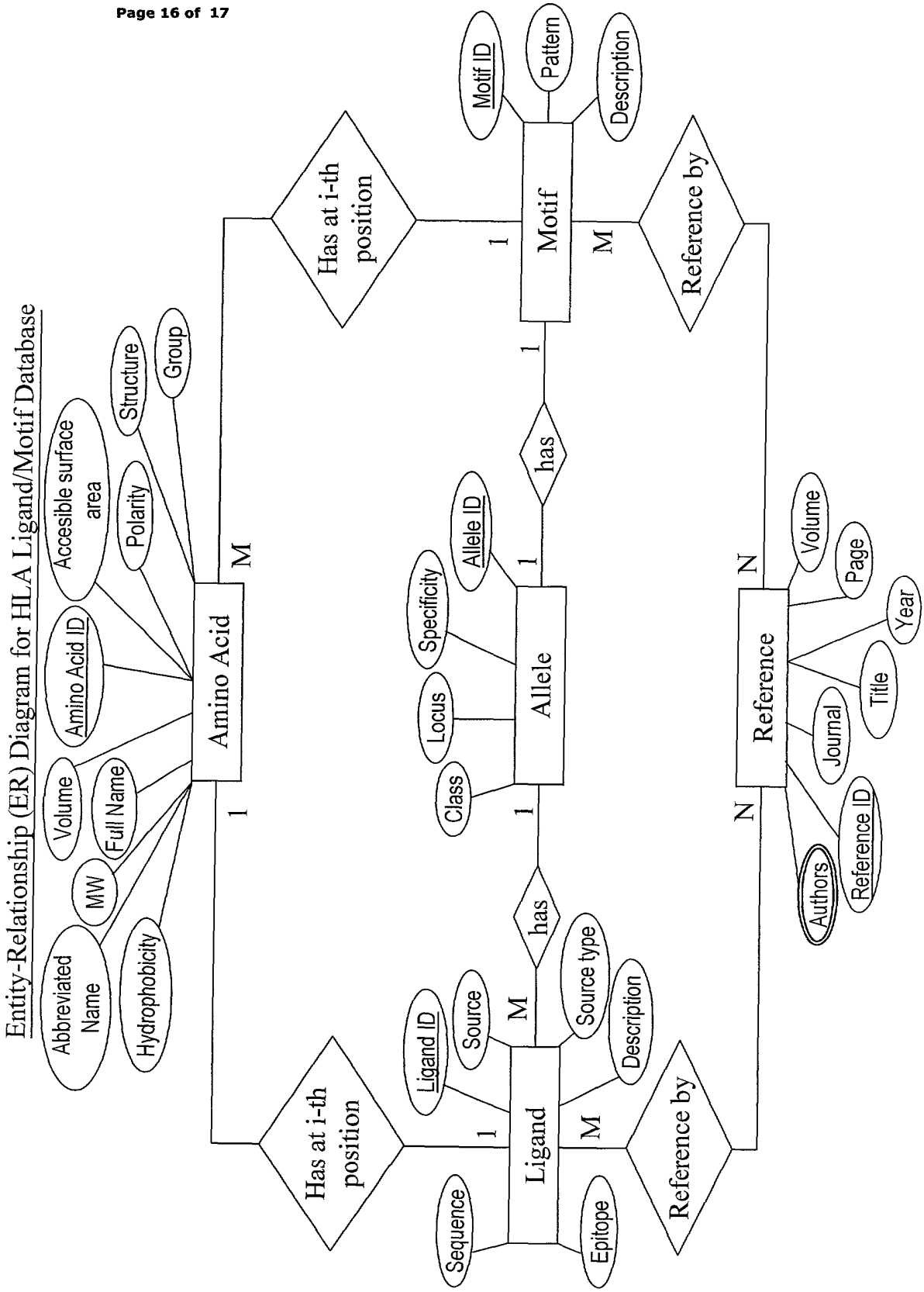


Fig. 13

20020090 "6023807

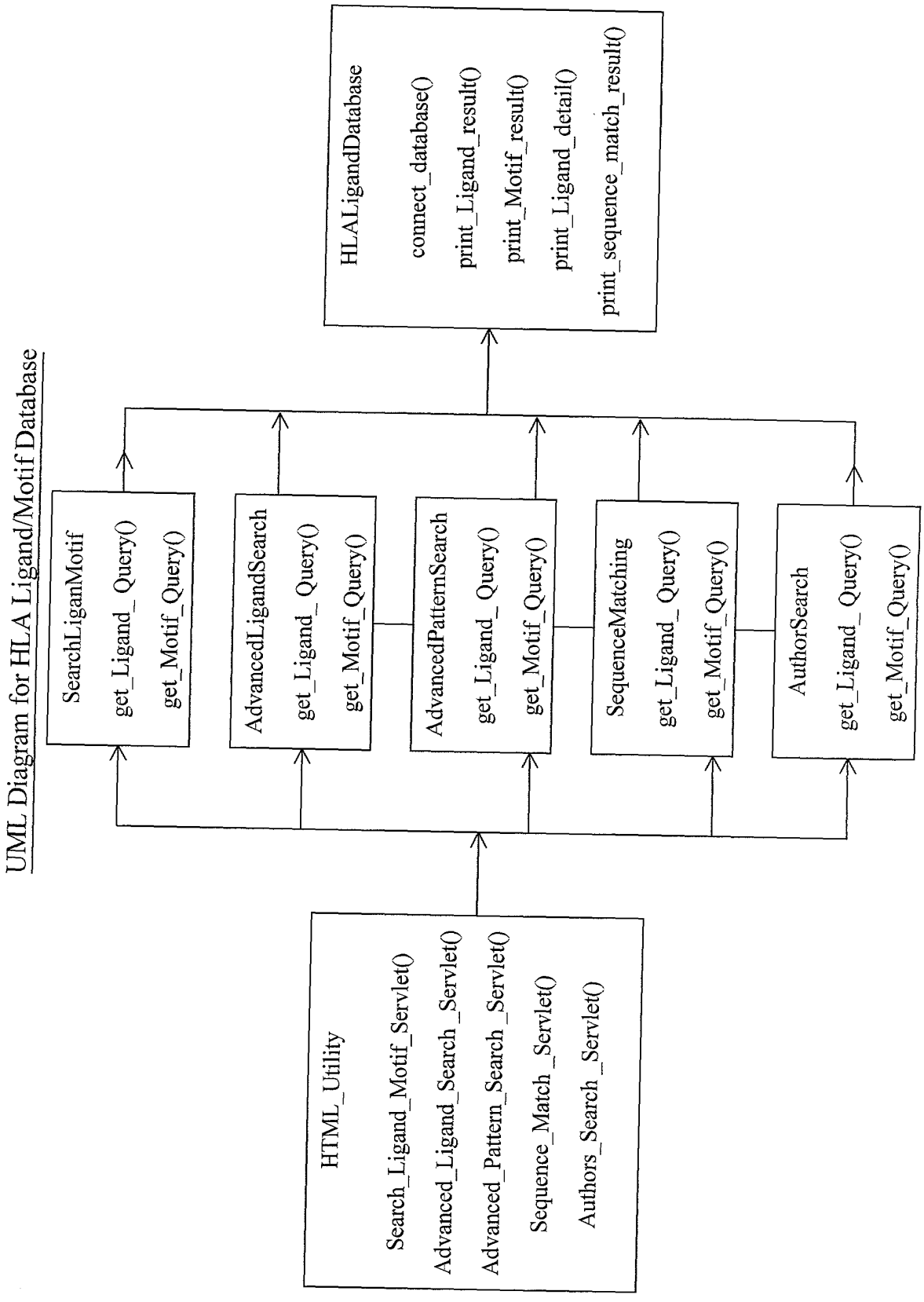


Fig. 14